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IONIC CONDUCTANCES IN GASTROINTESTINAL SMOOTH MUSCLES AND INTERSTITIAL CELLS OF CAJAL

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ABSTRACT

Ion channels are the unitary elements that underlie electrical activity of gastrointestinal smooth muscle cells and of interstitial cells of Cajal. The result of ion channel activity in the gastrointestinal smooth muscle layers is a rhythmic change in membrane potential that in turn underlies events leading to organized motility patterns. Gastrointestinal smooth muscle cells and interstitial cells of Cajal express a wide variety of ion channels that are tightly regulated. This review summarizes 20 years of data obtained from patch-clamp studies on gastrointestinal smooth muscle cells and interstitial cells, with a focus on regulation.

INTRODUCTION

The function of gastrointestinal smooth muscle is twofold: to mix intestinal content and thereby aid digestion, and to propel intestinal content. Specialized areas of the gastrointestinal tract also function as barriers (sphincters). For gastrointestinal smooth muscle to perform its function it must be able to contract and relax in synchronized patterns for the life span of the organism. Fundamental to the ability to contract is electrical excitability, with changes in smooth muscle contractile activity closely mirroring changes in smooth muscle membrane potential. The functional unit underlying the setting of the membrane potential is the ion channel. Ion channels are pores in the cell membrane

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that allow the rapid transfer of ions across the cell membrane. Ion channels are the elemental excitable unit in all cells, including smooth muscle. Individual ion channels can respond to a specific stimulus that can be electrical, such as a membrane potential change; chemical, such as a neurotransmitter; or mechanical. The response to a stimulus is an opening or closing of the pore, altering the permeability of the ion channel and changing the flux of ions across the membrane. Most ion channels are selective; that is, they have a selective permeability to a particular ion or class of ions. The main classes of ion channels are Na^+, K^+, Ca^{2+}, nonselective cation, and anion channels. This review summarizes our knowledge of the types of ion channels found in gastrointestinal smooth muscle and interstitial cells of Cajal, describes their biophysical properties and their regulatory mechanisms, and discusses their potential role in gastrointestinal physiology. I have elected to use information only obtained by direct measurement of whole-cell or single-channel currents. I have avoided detailed kinetic analysis of each channel type, as such information is best suited for more focused reviews. On the other hand, I include, whenever possible, experimental conditions and drug doses to aid experimentalists. Finally, I only discuss the regulatory pathways and biophysical properties of gastrointestinal smooth muscle ion channels, even if substantial relevant information exists outside of the gastrointestinal tract.

CALCIUM CHANNELS

Introduction

Calcium (Ca^{2+}) plays an important role in the function of all cell types but plays an even more central role in muscle cells. Ca^{2+} triggers contraction in smooth muscle and is a ubiquitous second messenger, transducing signals from a variety of compounds including neurotransmitters, hormones, and growth factors. Opening of Ca^{2+} channels results in a rapid rise in intracellular Ca^{2+}, with an often greater than tenfold increase in cytosolic Ca^{2+} concentration.

Channel Classification

The current classification of Ca^{2+} channels includes L-type (Ca_L, high threshold) channels, T-type (Ca_T, low threshold), N-type, P-type, Q-type, and R-type. P-, Q-, and R-type Ca^{2+} channels have not been described in gastrointestinal smooth muscle, and there is only one report of N-type-like Ca^{2+} channels, from longitudinal muscle cells of the rat ileum. Therefore, this section focuses on L- and T-type Ca^{2+} channels.

Most cell types that express T-type Ca^{2+} channels also express L-type Ca^{2+} channels. In general, the maximal amplitude of current generated by T-type Ca^{2+} channels is tenfold less than the current generated by L-type Ca^{2+} channels.

It is therefore important to separate the two currents to accurately determine the channel types present in gastrointestinal smooth muscle. Maneuvers to separate the two channel types include the following (modified from 1):

1. Holding voltage. T-type Ca^{2+} channels activate at more negative potentials than L-type Ca^{2+} channels and inactivate at more negative potentials. Maximal current is also reached at more negative potentials. Therefore, at a holding voltage of -100 mV, both channel types are available for activation, whereas at -40 to -50 mV, L-type Ca^{2+} channels are still mostly available for activation, and T-type Ca^{2+} channels are mostly inactivated.

2. Permeability. The whole-cell current recorded from L-type Ca^{2+} channels with Ba^{2+} as the charge carrier is several-fold larger than with Ca^{2+} as the charge carrier. The size of T-type calcium channel current is unchanged when external Ca^{2+} is replaced with Ba^{2+}.

3. Kinetics. T-type Ca^{2+} channels inactivate faster than L-type Ca^{2+} channels and deactivate slower.

4. Blockers and activators. 1,4-Dihydropyridines such as nifedipine and related compounds are relatively specific blockers, and BayK 8644 is a relatively specific activator for L-type Ca^{2+} channels at concentrations of 10 μM or less. T-type Ca^{2+} channels tend to be more sensitive to Ni^{2+} and less sensitive to Cd^{2+} than L-type Ca^{2+} channels.

L-TYPE Ca^{2+} CHANNELS

Introduction

L-type Ca^{2+} channels are required for gastrointestinal smooth muscle contractility. Addition of nifedipine, an L-type Ca^{2+} channel blocker, to intestinal smooth muscle strips results in cessation of contractile activity. L-type Ca^{2+} channels are needed to allow Ca^{2+} entry into smooth muscle cells to sustain contractile activity. Therefore, it is not surprising that L-type Ca^{2+} channels have been found in all regions of the gastrointestinal tract.

Channel Structure

L-type Ca^{2+} channels are made up of at least five subunits, α_1, α_2, β, δ, and γ. The α_1-subunit (195 kDa) is the major transmembrane component of the L-type Ca^{2+} channel. This subunit includes the ion conductance pore, and its expression alone is enough to form a channel that conducts Ca^{2+}. The α_2-subunit is a $\approx$28-kDa heavily glycosylated protein and in smooth muscle is highly conserved. In cardiac Ca^{2+} channels, coexpression of the $\alpha_2\delta$-subunit with the

α_1-subunit decreased activation time (2). β- and γ-subunits have not been cloned from gastrointestinal smooth muscle. Coexpression of the cardiac α_1-subunit and the brain β-subunit resulted in a marked increase in activation rate, a negative shift in the voltage of peak inward current, and an increase in peak inward current (reviewed in 1). No non-skeletal γ-subunit has been cloned.

Single Channel Conductance

An initial study on gastrointestinal L-type single channel Ca^{2+} currents found a 30-pS Ca^{2+} channel in cell-attached patches from guinea pig taenia coli with 50-mM Ba^{2+} as the charge carrier in the bath (3). Another study found, in cell-attached patches from guinea pig taenia coli, a 25-pS channel with 50-mM Ba^{2+} as the charge carrier in the bath and 140-mM Cs^{+} in the pipette solution, as well as a 12-pS Ca^{2+} channel (4). The 25-pS channel was blocked by nifedipine (2 μM), suggesting that it was an L-type Ca^{2+} channel. In contrast, the 12-pS channel was not affected by nifedipine, was not blocked by Cd^{2+} (10 μM in pipette), and exhibited rapid inactivation. The authors suggested that the smaller conductance channel may represent a T-type Ca^{2+} channel. A 20-pS nifedipine-sensitive Ca^{2+} channel was reported from smooth muscle cells of the rabbit ileum with 50-mM Ba^{2+} as the charge carrier (5), and 2 nifedipine-sensitive Ca^{2+} channels were identified from canine colonic circular smooth muscle cells with 80-mM Ba^{2+} as the charge carrier (6). One channel had a conductance of 10 pS and was infrequently seen, whereas the dominant channel had a conductance of 21 pS. At a molecular level, only the class C α_1-subunit and only one splice variant class C α_1-subunit, rbC-II, were present. A single-channel conductance of 17 pS was reported in canine and human jejunal circular smooth muscle cells, with 80-mM Ba^{2+} as the charge carrier (7). Nifedipine (1 μM) inhibited nearly all channel activity (Figure 1).

Whole-Cell Current Kinetics and Regulation

The whole-cell current that results from the opening of any single channel conductance, including single Ca^{2+} channels in smooth muscle cells, can be modeled as:

$$I = N \cdot i \cdot P_o,$$

where I is the whole-cell current, N the number of channels in the cell membrane, i the unitary current through a single channel, and P_o the probability that a channel is open. P_o is voltage dependent and can be subdivided into the probability that a channel is available to be opened and the probability that a channel that is available to be opened is open. The relationship between steady-state activation and inactivation gives rise to the whole-cell window current, i.e. the current that would be expected to flow under steady-state conditions at a

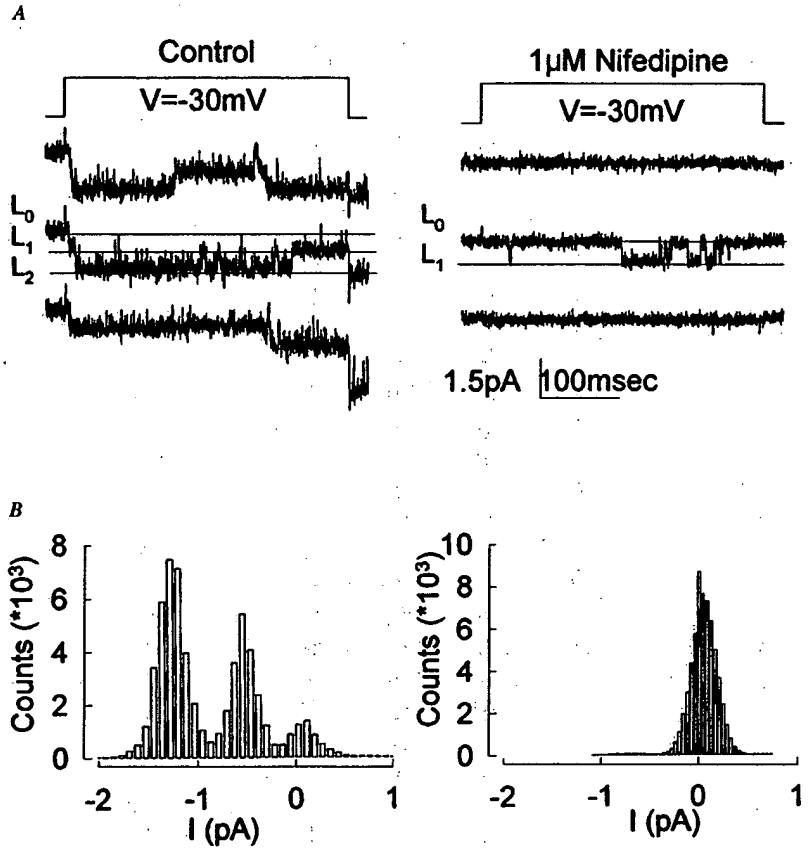

Figure 1 L-type Ca^{2+} channel activity recorded from a canine jejunal circular smooth muscle cell bathed in 150 mM KCl and 80 mM Ba^{2+} as the charge carrier. Single channel activity recorded from an on-cell patch before (*A*) and after nifedipine (*B*). At least two channels are present in this patch (L_0 = closed, L_1 = 1 channel, L_2 = 2 channels). As can be seen from the histograms, nifedipine blocked nearly all channel activity (NP_o = 0.99 before, NP_o = 0.0004 after nifedipine) (7).

particular voltage. The window current is useful as it allows assumptions to be made on the size of the steady-state Ca^{2+} current at a particular voltage, such as at the resting membrane potential of a gastrointestinal smooth muscle cell and the change that can be expected if the cell membrane potential were to change. The voltage dependence of activation and inactivation can be fit by a Boltzmann equation:

$$I/I_{max} = 1/(1 + \exp[V_h - V]/k),$$

where I is the current, I_{max} the maximal inward current, V is the test activating potential, V_h the test potential eliciting half-maximal I, and k the slope factor. The resulting curve is sigmoidal. Steady-state inactivation can also be fit by a Boltzmann equation, again resulting in a sigmoidal curve. In canine jejunal circular smooth muscle cells, steady-state activation was fit by a single Boltzmann curve with a V_h of -6 mV and a k of -5.7 mV (7). Inactivation was also fit with a V_h of -36 mV and a k of 14 mV. The two curves resulted in a peak window current at about -15 mV. The records were obtained with 80 mM Ba^{2+} as a charge carrier. The voltage sensed by L-type Ca^{2+} channels is modified by the surface charge screening effects of cations in the bath solutions. In canine and human jejunal circular smooth muscle cells, 80 mM Ba^{2+} results in a shift in the activation-voltage curve in the depolarization direction of about 25 mV.

The temperature-dependence of the L-type Ca^{2+} channel current in gastrointestinal smooth muscle appears to be similar to that reported from other smooth muscle cells. The amplitude of the inward current carried by Ba^{2+} increases by $\approx$3 in ventricular myocytes for a 10°C rise in temperature (Q_{10}) (8) and by 2.1–2.4 in skeletal muscle. In guinea pig taenae coli myocytes, a Q_{10} of 1.7 (9) was reported and in canine jejunal circular smooth muscle cells, the reported Q_{10} was $\approx$2.1 (10).

RUNDOWN L-type Ca^{2+} channel current runs down during traditional whole-cell patch-clamp recordings. Rundown may be preceded by a brief run-up period where the peak inward current increases over a short time period ($\approx$5 min), suggesting that it is secondary to wash out of cytoplasmic contents that suppress L-type Ca^{2+} channel current. The mechanism of rundown is unclear. Contributing factors include the effects of entry of cations, such as Ca^{2+}, on intracellular phosphatases or proteases. In non-smooth muscle cells, Ca^{2+}-dependent protease inhibitors reduce rundown. Phosphatase inhibitors may also reduce rundown. Calyculin A, an inhibitor phosphatase types 1 and 2A, induces an increase in inward current recorded from canine jejunal circular smooth muscle cells (11). In a variety of cell types, including gastrointestinal smooth muscle (12), intracellular ATP reduces rundown probably by several mechanisms including favoring phosphorylation, Ca^{2+} regulation, and protecting Ca^{2+} channels from enzymatic hydrolysis (1).

In excised patches, rundown is about tenfold faster than rundown of whole-cell currents, which makes it difficult to record L-type Ca^{2+} channel current in excised patches. As in whole-cell currents, potential mechanisms include dephosphorylation, proteolysis, and change in intracellular Ca^{2+} and G-protein concentrations. There may be differences between the mechanisms of rundown in gastrointestinal smooth muscle cells and cardiac smooth muscle cells. In canine colonic smooth muscle cells, addition of BayK 8644 (1 μM) and EGTA (5 mM) to the bath enables prolonged single-channel recordings from

excised patches (6), whereas a similar approach was unsuccessful in guinea pig ventricular cells (13).

CALCIUM Chelation of intracellular Ca^{2+} with EGTA or BAPTA reduces inactivation, which suggests that a rise in intracellular Ca^{2+} increases inactivation of L-type Ca^{2+} channels. More evidence for the role of intracellular Ca^{2+} in altering the behavior of L-type Ca^{2+} channels is the marked reduction in inactivation when Ba^{2+} is substituted for Ca^{2+} as the charge carrier, while peak inward current is increased. This suggests that L-type Ca^{2+} channels are more permeable to Ba^{2+} than to Ca^{2+} and that Ca^{2+} stimulates inactivation. For example, in guinea pig gastric myocytes (14) with Ca^{2+} as the charge carrier, inactivation was fitted with two exponentials of $\tau \approx 53$ and $\tau \approx 175$ ms. With Ba^{2+} as the charge carrier, inactivation was well fit with one exponential of $\tau \approx 145$ ms, suggesting that the fast inactivation time constant was Ca^{2+} dependent.

Ca^{2+}-dependent enhancement of Ca^{2+} current has been reported in gastrointestinal smooth muscle cells from the stomach of *Bufo marinus*. In these gastric myocytes, two components of the inward Ca^{2+} current were identified, a low- and a high-voltage-activated component. The high-voltage-activated component (presumably L-type) was enhanced by raising intracellular Ca^{2+}. The increase in current was maximal with an internal Ca^{2+} of $\approx$600 nM (15).

pH L-type Ca^{2+} channel activity in most cell types is inhibited by extracellular acidification and enhanced by extracellular alkalization. In guinea pig gastric myocytes (14), a change in extracellular pH from 7.2 to 8.0 increased maximal inward current by $\approx$70%, and a change in pH from 7.2 to 6.0 decreased maximal inward current by $\approx$71%. Potential mechanisms of the effects of pH include neutralization of surface charge, block by external protons, and block by internal protons.

cAMP AND PHOSPHORYLATION The effects of cAMP in gastrointestinal smooth muscle appear to be diverse. cAMP was without effect on L-type Ca^{2+} channels in guinea pig ileal smooth muscle cells (12), and phenylephrine in rat anococcygeus muscle produced a dose-dependent inhibition of L-type Ca^{2+} channel current (16). In canine colonic myocytes (17), low concentrations of cAMP activated L-type Ca^{2+} channel current and higher concentrations inhibited the current.

The effects of phosphorylation on L-type Ca^{2+} channel current have also been tested in gastrointestinal smooth muscle. Yabu et al reported that calyculin A increased inward current through L-type Ca^{2+} channels (carried by Ba^{2+}) from guinea pig taenia coli smooth muscle, which suggested that phosphorylation increased inward current in these cells (18). Ward et al studied L-type Ca^{2+} channel current recorded from colonic and gastric canine smooth muscle cells (19). In contrast to the above results, in traditional whole-cell

patch-clamp experiments both okadaic acid and calyculin A reduced peak inward current. Calyculin also contracted the isolated smooth muscle cells and raised intracellular Ca^{2+} measured with indo-1. Okadaic acid did not cause gastric cell contraction or a change in intracellular Ca^{2+}, which led the authors to speculate that the apparent rise in intracellular Ca^{2+} observed with calyculin A may have been secondary to changes in cell shape. Decrease of L-type Ca^{2+} channel current with okadaic acid was also reported by Lang et al (20). A possible explanation for the apparently discordant results is that Yabu et al used Ba^{2+} as the charge carrier and Ward et al used Ca^{2+}. Ca^{2+} may have direct effects on L-type Ca^{2+} channels or modify other regulatory mechanism that control L-type Ca^{2+} channels. Another possible explanation was put forward by Obara & Yabu in 1993 (21). The authors found that low concentrations of okadaic acid (10 nM) and calyculin A (0.5 nM) decreased inward current in guinea pig taenia coli smooth muscle cells, whereas higher concentrations increased inward current. The authors suggested that the inhibitory effects were mediated through type 2A protein phosphatases and the stimulatory effects through type 1 protein phosphatases. The effects of phosphorylation on L-type Ca^{2+} current were also studied in isolated canine jejunal circular smooth muscle cells with Ba^{2+} as the charge carrier (11). G-protein stimulation via GTP-γS increased inward current, whereas G-protein inhibition via GDP-βS inhibited the inward current. The G-protein effects appeared to be mediated by a cholera toxin–sensitive, pertussis toxin–insensitive G protein. Activation of the L-type Ca^{2+} channel current by G proteins appeared to be secondary to a change in protein phosphorylation as staurosporine, a nonspecific protein kinase inhibitor, inhibited the effects of G-protein activation on inward current and calyculin A accentuated the effects of G-protein activation on inward current. Calyculin A (100 nM) had a biphasic action on L-type Ca^{2+} channel current, first activating, then inhibiting L-type Ca^{2+} channel current, which again suggested a dual effect of phosphorylation in the modulation of L-type Ca^{2+} channel current in gastrointestinal smooth muscle.

OTHER REGULATORY MECHANISMS Muscarinic receptors are found on gastrointestinal smooth muscle cells, and the receptors are coupled to a variety of G proteins. Both an inhibition of L-type Ca^{2+} channel current and an activation of L-type Ca^{2+} channel current by ACh can be predicted depending on which G-protein cascade is activated. In 1985, Sims et al (22) recorded currents from toad gastric smooth muscle and demonstrated activation of Ca^{2+} channels and inhibition of K^+ channels in response to ACh. ACh (1 μM) and erythromycin (1 μM), a promotility agent, activated L-type Ca^{2+} channel current (carried by Ba^{2+}) in canine jejunal circular smooth muscle cells (7). The increase in current was blocked by nifedipine, which suggests that it was carried by L-type Ca^{2+} channels. In contrast, ACh was found to decrease L-type Ca^{2+} current in

other gastrointestinal myocytes such as guinea pig gastric myocytes (23) and canine colonic myocytes (24).

L-type Ca^{2+} channel current in human and canine jejunal circular smooth muscle cells is also modulated by the gastrointestinal hormone motilin (25). Motilin increased L-type Ca^{2+} channel current, carried by Ba^{2+}, in a dose-dependent manner. The effects of motilin appeared to be mediated through a pertussis toxin–insensitive G-protein pathway.

T-TYPE Ca^{2+} CHANNELS

Introduction

T-type Ca^{2+} channels are found in a several smooth muscle cells including vascular and cardiac smooth muscle. T-type Ca^{2+} channels have been described in gastrointestinal myocytes of the guinea pig, rat, and *Bufo marinus*. Their physiological role is not as clear as that for L-type Ca^{2+} channels. T-type Ca^{2+} channels are generally active at voltages negative to -60 mV and inactivate rapidly at more depolarized voltages. In smooth muscle cells with membrane potentials positive to -60 mV, little current is expected to flow through T-type Ca^{2+} channels. In cardiac sino-atrial cells, T-type Ca^{2+} channels have been implicated in the generation of the pacemaker current (26), and a similar role for T-type Ca^{2+} channels has been proposed in gastrointestinal smooth muscle cells and in interstitial cells of Cajal (see below).

Channel Structure

The T-type Ca^{2+} channel has not yet been cloned in gastrointestinal smooth muscle and little information is known about the channel structure. Recently, a neuronal T-type channel has been cloned and expressed. The single channel conductance in *Xenopus* oocytes was 7.5 pS (27).

Single Channel Conductance

Yoshino et al (28, 29) reported two Ca^{2+} channel types in smooth muscle cells of guinea pig taenia coli. One fulfilled the criterion for an L-type Ca^{2+}. The other had a conductance of 12 pS, inactivated rapidly, was inactivated at a holding potential of -40 mV, and was insensitive to nifedipine (2 μM). The 12-pS channel openings were mostly seen at the beginning of depolarizing pulses. The data suggest that the 12 pS channel was a T-type Ca^{2+} channel. The authors, however, also pointed out that the activation threshold for the 12-pS channel was -40 mV, a depolarized value compared with cardiac T-type Ca^{2+} channels.

Whole-Cell Current Kinetics and Regulation

Yoshino et al (28, 30) recorded whole-cell and single-channel currents from guinea pig taenia coli myocytes. Whole-cell current inactivation was best fit

with two exponentials, one thought to represent the fast inactivating (T-type) Ca^{2+} channel current, insensitive to nifedipine, and one thought to represent the slow inactivating (L-type) Ca^{2+} channel current, sensitive to nifedipine. Voltage dependence of activation was similar for both components. Yamamoto et al (31) also studied inward Ca^{2+} current from guinea pig taenia coli myocytes. In their study, inactivation was best fit by three exponentials of 7, 45, and 400 msec, at 0 mV and 33°C. Activation was best fit with a single exponential. The authors suggested that the inactivation characteristics of the Ca^{2+} current reflect the presence of more than one Ca^{2+} channel type. Katzka & Morad (14) recorded whole-cell inward currents from guinea pig gastric myocytes. Inactivation was best fit with two exponentials; however, the authors found little evidence for two Ca^{2+} channel types as inward current activated at a relatively depolarized −20 mV; changing the holding voltage did not alter inactivation kinetics, as would be expected if a T-type Ca^{2+} channel was present, and nifedipine did not preferentially block a component of the Ca^{2+} current.

There also seems to be a difference in the relative expression of the putative two channel types with age (32). Two components of inward Ca^{2+} current were recorded from newborn and adult rat ileal myocytes. In newborn rat ileal myocytes (1–3 days old), two components of inward Ca^{2+} (carried by 10 mM Ca^{2+}) current were observed (apparently in all 94 cells studied). One activated at a voltage range of ≈40 mV, had a fast inactivation rate, and was completely inactivated at a holding voltage of −50 mV. The other component activated at ≈−10 to −20 mV, had a slower inactivation, and was well discernible at a holding voltage of −50 mV. Of interest, nifedipine (1 μm) had no effect on the two components of the inward current in newborn ileal myocytes. Nifedipine (30 μM) blocked ≈20% of peak inward current with no effect on the low-voltage activating component (presumably T-type) of the inward current. Cadmium (10 μM) blocked the high-voltage activating component (presumably L-type) with no effect on the low-voltage activating component. Cadmium (100 μM) blocked both components, whereas nickel (500 μM) blocked the low-voltage activating component with little effect on the high-voltage activating component. In contrast, in the adult rat (2.5–3 months), only a high-voltage activating component of the inward Ca^{2+} current was seen with a greater sensitivity to nifedipine than to the current (as in newborn rats). In several of the 16 cells studied, a fast inactivating component of the inward Ca^{2+} current was observed but was too small to study. The authors conclude that in the ileal myocytes of newborn rats a T-type Ca^{2+} channel is present, as well as another channel type suggestive of both L- and N-type Ca^{2+} channels and with a low sensitivity to nifedipine. In adult rat ileal myocytes, T-type Ca^{2+} channels are absent or present in very small numbers.

Age-dependent changes in inward current were also recorded from rat colonic myocytes from 1–820 days old (33). At a holding potential of −100 mV two

components of the current were observed. The T-type component activated at about −60 mV, had a half-life of inactivation of <100 ms, was resistant to nifedipine (2–10 μM) and cadmium (20 μM), and was blocked by nickel (30 μM). The L-type component activated at about −40 mV, had a half-life of inactivation of 200 ms, was blocked by nifedipine and cadmium, but not by nickel. The L-type Ca^{2+} channel current was present in all cells of all ages studied. In contrast to the findings in the rat ileum, the T-type Ca^{2+} channel current was not present in newborn rats (1 day), was present in 50% of cells at day 30 and in 70% of cells at day 480. The current density for the T-type Ca^{2+} channel increased until day 30 where it remained stable, whereas the current density for the L-type Ca^{2+} channel increased until day 30 but declined after day 120.

In canine colon (6, 34) and in human and canine jejunum (7), only L-type Ca^{2+} channels were identified. The T-type Ca^{2+} channel current is voltage sensitive and inactivates at depolarized voltages. In the jejunum a holding voltage of −70 mV was used, whereas in the rat colon a holding voltage of −100 mV was used. In the canine colon two single channel conductances were identified; one had a conductance of ≈12 pS, which would be similar to that reported for other T-type Ca^{2+} channels. However, the channel was blocked by nifedipine (10 μM), did not exhibit rapid steady-state inactivation at depolarized voltages, and had long open times, which suggested it was not a T-type channel. The presence of T-type Ca^{2+} channels in gastrointestinal myocytes in species other than rat, *Bufo marinus*, and guinea pig therefore remains to be established. Regulatory pathways affecting T-type Ca^{2+} channels have not been studied in gastrointestinal myocytes.

Of interest, a non L-type, non T-type Ca^{2+} channel was recently identified in mouse duodenal myocytes (35). The whole-cell Ca^{2+} current was blocked by mapacalcine, a toxin derived from the marine sponge *Cliona vastifica*. Mapacalcine had no effect on T-type Ca^{2+} channels in portal vein myocytes and did not interact with the dihydropyridine receptor, suggesting a new Ca^{2+} current was identified. The occurrence of this current in other species remains to be identified.

RUNDOWN In cells where rundown of T-type Ca^{2+} channel current has been studied, little rundown has been observed (1).

CHLORIDE/ANION CHANNELS

Introduction

Anion channels are often referred to as Cl^- channels, although most are also permeable to other anions such as Br^- and I^- and often also variably permeable to larger anions. In the following section the term Cl^- channel is used

interchangeably with anion channel. Under recording conditions that are close to physiological ionic conditions, opening Cl^- channels usually results in movement of Cl^- out of the cell and results in membrane depolarization toward the equilibrium potential for Cl^-. If the membrane potential is depolarized beyond Cl^- equilibrium potential, as can occur during a contraction, then opening of Cl^- channels results in Cl^- influx and membrane hyperpolarization. As the equilibrium potential for Cl^- is not far from the cell membrane potential, opening of Cl^- channels generally does not lead to large changes in membrane potential.

Channel Classification and Structure

Chloride channels can be classified, based on their mechanism of activation, into voltage-gated Cl^- channels, Ca^{2+}-activated Cl^- channels, ligand-gated Cl^- channels, mechanosensitive Cl^- channels, G protein-regulated Cl^- channels, and volume-gated Cl^- channels. Also in this classification is the cystic fibrosis transmembrane conductance regulator (CFTR) channel, which is a cyclic AMP-activated Cl^- channel. Considerable overlap exists between the groups as, for example, Ca^{2+} activated channel Cl^- channels are usually also voltage regulated, and volume-regulated Cl^- channels are often also regulated by messengers and voltage. An alternative classification is one based on structure of cloned Cl^- channels (adapted from 36). There are four broad classes of Cl^- channels: (*a*) ligand-gated anion channels, such as the glycine receptor and the $GABA_A$ receptor, which are assembled as pentamers with each monomer having 4 transmembrane spans; (*b*) CLC Cl^- channels, which include voltage-gated Cl^- channels, as well as volume-activated channel Cl^- channels such as CLC-2 and the recently cloned CLC-3, thought to represent a ubiquitous volume regulated Cl^- channel (50); channels in this family have about 12 transmembrane spans; (*c*) The CFTR channel, which is a cyclic AMP-activated channel highly selective for Cl^-, and whose mutations lead to cystic fibrosis; this channel has 12 putative transmembrane spans and may also function as an ion channel regulator; (*d*) Ca^{2+}-activated Cl^- channels, although controversy still exists on whether truly Ca^{2+}-activated Cl^- channels have been cloned. Other cloned proteins such as pl_{Cln} have been claimed to function as Cl^- channels but because controversy exists on their function and they have not been identified in gastrointestinal myocytes, they are not be dealt here.

Ca^{2+}-ACTIVATED Cl^- CHANNELS

Single Channel Conductance

Application of neurokinin (NK) agonists resulted in Ca^{2+} oscillations and activation of Cl^- channels in on-cell patches of rabbit longitudinal colonic

myocytes. The Cl^- channel conductance was not stated, but based on a figure in Reference 37, it appears to be ≈20 pS.

Whole-Cell Current Kinetics and Regulation

In gastrointestinal myocytes, Ca^{2+}-activated Cl^- channels were first described by Byrne & Large in rat anococcygeus muscle (39). Application of the Ca^{2+} ionophore A23187(1 μM) or caffeine (10 mM) resulted in an increase in Cl^- whole-cell current.

Carbachol stimulates a Cl^- channel in rat small intestinal smooth muscle cells. The increase in Cl^- conductance was accompanied by a rise in intracellular Ca^{2+} and blocked by intracellular heparin but not by EGTA (2 mM), suggesting it was, in part, regulated by Ca^{2+} released from $InsP_3$-sensitive stores (40). A second Cl^- conductance was also activated by caffeine (10 mM) but was abolished by intracellular EGTA (2 mM) and extracellular ryanodine (20–50 μM), suggesting that it was regulated by ryanodine-sensitive Ca^{2+} stores (41).

A Ca^{2+}-activated Cl^- current was also described in rabbit esophageal muscularis mucosae myocytes (42). In whole-cell recordings (Cs^+ in the pipette to block K^+ currents), depolarization evoked inward Ca^{2+} currents and an outward current. The outward current was inhibited when the pipette contained EGTA or the bath contained Ba^{2+}, suggesting Ca^{2+} dependence. The reversal potential of the outward current changed in accordance to the equilibrium potential for Cl^-, and the whole-cell current was blocked by niflumic acid (10 μM), suggesting it was carried by Cl^-. Increase in intracellular Ca^{2+} activates a Cl^- conductance in opossum esophageal circular myocytes (43). Caffeine (10 mM) and carbachol (10 μM) contracted the myocytes and activated a whole-cell current that was predominantly carried by Cl^-. Ionomycin (10 μM) activated both the Cl^- current and a nonselective cation current. As the inhibitory junction potential in opossum esophageal circular smooth muscle is Cl^- dependent (44), it appears that Cl^- channels have an important role in esophageal electrophysiology of the opossum.

In canine jejunal circular smooth muscle cells (45), increases of intracellular Ca^{2+} by cyclopiazonic acid or thapsigargin activated a Cl^- conductance. Activation of the Cl^- conductance was prevented by Bapta (10 mM), suggesting the presence of a Ca^{2+}-activated Cl^- conductance.

VOLTAGE- AND SECOND MESSENGER-REGULATED Cl^- CHANNELS

Single Channel Conductance

A 300 pS Cl^- channel (140 mM Cl^- pipette, 126 mM Cl^- bath) was identified in rabbit colon longitudinal myocytes (46, 47). The Cl^- channel was

voltage dependent with a bell-shaped voltage activation curve. Maximal open probability was at −5 to +20 mV. Several subconductance states were observed, with a smallest subconductance state of 15 pS. The channel was not activated by Ca^{2+} (up to 1 mM) and was blocked by DIDS and NPPB. In inside-out patches, GTP-γS (nonhydrolyzable GTP analog) activated the channel within 30 sec and the inhibitor of G-protein activation, GDP-βS, reversibly inhibited channel activity. G protein-activation of the 300 pS Cl^- channel appeared to be via a pertussis toxin-sensitive G protein. The Cl^- channel was also activated by the NK-1 receptor agonist substance P methylester but not by protein kinase A or C.

Whole-Cell Current Kinetics and Regulation

The Ca^{2+}-activated Cl^- channels from rat anococcygues muscle (39) were also activated by noradrenaline (applied by ionophoresis) and by voltage. Noradrenaline activated a whole-cell current that was blocked by phentolamine.

A Ca^{2+}-activated Cl^- channel was also found in opossum esophageal circular smooth muscle cells (43). This Cl^- current was also activated by carbachol (10 μM), most likely through a rise in intracellular Ca^{2+}. Carbachol regulation of Cl^- currents may play a role in esophageal contractile activity, as explained above.

VOLUME-REGULATED Cl^- CHANNELS

Single Channel Conductance

Single channel conductances have not been reported for gastrointestinal volume-regulated (activated) Cl^- channels.

Whole-Cell Current Kinetics and Regulation

Volume-activated anion channels are found in most, if not all, mammalian and nonmammalian cell types. The channels are thought to contribute to cell-volume regulation and may also play a role in setting the membrane potential (48), as they are often active under baseline conditions. Volume-activated Cl^- channels also participate in intracellular pH regulation, epithelial Cl^- transport and fluid secretion, exocytosis, transmembrane transport of organic osmolytes (including amino acids), and cell proliferation.

In guinea pig gastric antral myocytes (49), osmotic cell swelling resulted in activation of a volume-activated anion current sensitive to DIDS (100 μM) and niflumic acid (10 μM). Arachidonic acid (25 μM) also inhibited the current. Use of indomethacin (25 μM) and chelerythrine (1 μM), cyclooxygenase, and protein kinase C (PKC) inhibitors, respectively, did not influence the volume-activated anion current or the effects of arachidonic acid. The anion

permeability sequence for the anion current was $I^- > Br^- > Cl^- >$ gluconate. The current-voltage relationship showed outward rectification. The properties of this current are similar to those of a volume-regulated anion current attributable to ClC-3, a Cl^- channel recently cloned from guinea pig heart (50). However, unlike ClC-3, the volume-activated anion current in guinea pig antrum was not inhibited by PKC.

A volume-activated anion current was recently described in human and canine jejunal circular smooth muscle (51, 52). In both human and canine myocytes, the current was activated by osmotic cell swelling (212 mOSM). The volume-activated Cl^- current was not blocked by DIDS, 9-AC, ketoconazole, or tamoxifen (100 μM for all), nor was it inhibited by PKC activation (500 nM). The anion selectivity of the volume-activated current was $I > Br > Cl > F$. The data suggest the presence of a volume-activated Cl^- current in human and canine jejunal circular smooth muscle cells with properties different from those of CLC-3 and the guinea pig gastric myocyte volume-activated anion current, suggesting that these currents are carried by a different Cl^- channel.

NONSELECTIVE CATION CHANNELS

Introduction

Nonselective cation (NSC) channels are ion channels that are equally permeable to Na^+ and K^+ and thus have a reversal potential near 0 mV. Under physiological ionic gradients, opening of NSC channels results predominantly in Na^+ influx and membrane depolarization.

Channel Classification

NSC channels were the first channels to be described. In 1951, Fatt & Katz described a large nonselective increase of ion permeability induced by ACh at the muscle end plate (53). NSC channels encompass a large group of channels, ranging from the nicotinic ACh receptor to gap junctions. The group includes ligand-gated NSC channels, mechanosensitive NSC channels, the cGMP-gated NSC channel involved in olfactory and visual signal transduction, bacterial porins, and gap junction NSC channels, Ca^+-regulated NSC channels, ATP-gated NSC channels, and receptor-regulated NSC channels. In gastrointestinal myocytes, only receptor and Ca^{2+}-regulated NSC channels have been described, and this section is limited to these groups. The term NSC channel is used interchangeably with receptor and Ca^{2+}-regulated NSC channels.

Channel Structure

NSC channels that express currents similar to those found in gastrointestinal myocytes have not been cloned yet. The structure of the nicotinic ACh receptor,

a NSC channel, is well understood; however, it is not known if nicotinic ACh receptors are found in gastrointestinal myocytes.

Single Channel Conductance

Inoue et al (54) determined the conductance of an ACh-activated NSC channel from guinea pig ileal longitudinal myocyte on-cell patches. The channel conductance was 20–25 pS, with a Na^+/K^+ permeability ratio of 1/0.3–0.4. The channel was voltage dependent with a bell-shaped current-voltage relationship. Similar experiments were performed on canine gastric pyloric myocytes (55). ACh (10 μM) activated a NSC channel with a unitary conductance of 30 pS.

In cultured guinea pig ileum longitudinal smooth muscle cells, a 12 pS NSC channel was identified (inside-out patches, 150 mM KCl in pipette and bath). The channel was voltage dependent with half-maximal activation at $\approx$7 ms and a 60 mV change required to increase P_o *e*-fold. The permeability ratios (P_x/P_K) for Na^+, Ca^{2+}, and Li^+ were 1, 0.86, and 1.17, respectively. The NSC channel was insensitive to Ca^{2+} (1 mM) and was blocked by Cs^+ and Ba^{2+}. Angiotensin II (100 nM) increased P_o, an effect blocked by the angiotensin II antagonist Sar^1-Leu^8-Ang II (200 nM) (56).

An 80 pS NSC channel was identified from *Bufo marinus* gastric myocytes (on-cell patches 120 NaCl bath, 130 CsCl pipette). The channel was insensitive to voltage from −60 to +60 mV, activated by caffeine, and permeable to Ca^{2+} in the presence of extracellular Na^+ (56a).

Whole-Cell Current Kinetics and Regulation

Two types of whole-cell NSC currents have been described in the gastrointestinal tract. One is a baseline current that is present under unstimulated conditions, and the other is a NSC current induced by activation of muscarinic receptors. The currents are similar and may represent degrees of activation of the same channels. There may, however, be more than one type of NSC current in myocytes. In canine pyloric myocytes (55), ACh-activated a NSC current that was more noisy than the baseline current and was blocked by Cs^+, which is usually permeable through NSC currents. P_K/P_{Na} of the ACh-activated current was 1.7. The data suggest that ACh-activated a second NSC current.

Two currents carried by external Na^+ (presumably NSC currents) were recorded from longitudinal myocytes of guinea pig ileum (57). One current was activated by ACh (10 μM) and relaxed to a steady state within 200 ms of activation. The other was activated by substance P (SP), did not relax over a longer time period, and increased in size with hyperpolarization. The difference in inactivation time constants, voltage dependence, and differential response to SP and ACh led the authors to suggest the presence of two separate currents. Also, in canine colonic myocytes, substance P and neurokinin A, on the one

hand, and Ach, on the other hand, activated NSC currents with different I-V relationships (58).

A NSC current in gastrointestinal myocytes was first described by Benham et al in 1985 in rabbit jejunal longitudinal myocytes (59). The current-voltage relationship obtained from the whole-cell NSC current was U shaped, with an increase in inward current at about −40 to −30 mV and then a decrease in current as the current direction changes to an outward direction at about 0 mV. The U-shaped current-voltage relationship may reflect a voltage dependence of the open probability combined with a change in driving force, with a larger driving force at −30 mV compared with no driving force at 0 mV. It may also be due to Ca^{2+} effects. However, nifedipine did not affect the shape of the current-voltage relationship, which supports the former explanation (60). Steady-state activation curves were obtained for longitudinal myocytes from guinea pig ileum (61). The ACh-activated NSC current had a half-maximal activation voltage of −58 mV.

BLOCKERS Chen et al (62) determined the effects of numerous ion-channel blockers on NSC current from longitudinal myocytes of guinea pig ileum. Cesium aspartate was used in the internal solution to inhibit K^+ currents, and ACh (10 μM) was used to stimulate the NSC current. The K^+ channel blockers, TEA (10 mM), procaine (5 mM), 4-AP (5 mM), and quinine (10 μM), inhibited the current by ≈45, 90, 75, and 90%, respectively (at a holding potential of −50 mV). Caffeine (10 mM), known to release Ca^{2+} from intracellular stores, inhibited the current by 45%, most likely through a direct effect on the NSC channel. The Ca^{2+}-activated NSC channel blockers, flufenamic acid, niflumic acid, and DCDPC, blocked the NSC current with ≈IC_{50}s of 32, 100, and 30 μM. These results point to the need for care in interpreting pharmacological experiments on whole-cell currents made up of a mixture of channel types, as the blockers may not be specific. Similar results were obtained by Kim et al (63). In this study, quinidine, 4-AP, and TEA inhibited NSC current in guinea pig gastric myocytes. The IC_{50}s for 4-AP and TEA were 3.3 and 4.1 mM, respectively. Quinidine at 2 μM appeared to be a somewhat specific blocker for the carbachol-induced NSC current, blocking ≈11% of inward Ca^{2+} current, ≈15% of the voltage-dependent K^+ current (at 30 mV), and ≈11% of the Ca^{2+}-activated K^+ current (at 45 mV) compared with near-complete block of the carbachol-induced NSC current.

CALCIUM The NSC current recorded from several regions of the gastrointestinal tract is Ca^{2+} regulated but not directly Ca^{2+} activated. The ACh-activated NSC is sensitive to internal Ca^{2+}, although the NSC current itself does not appear to be directly activated by a rise in intracellular Ca^{2+}. An increase

in intracellular Ca^{2+} to ≈ 1 μM in most gastrointestinal myocytes does not markedly alter the size of the NSC (64), but the response to ACh is markedly enhanced by a rise in intracellular Ca^{2+}. In voltage-clamp experiments where depolarization was used to elicit Ca^{2+} entry through Ca^{2+} channels, the effects of ACh on the NSC current were enhanced (65, 66). This enhancement was blocked by L-type Ca^{2+} channel blockers such as nifedipine and nitrendipine (65) and by replacing extracellular Ca^{2+} with Ba^{2+} or Sr^{2+}, suggesting it was specifically due to Ca^{2+} entry from L-type Ca^{2+} channels. Release of Ca^{2+} from intracellular stores may also be involved in the enhancement of the ACh-activated NSC current by Ca^{2+}. If caffeine or ryanodine is used to deplete intracellular Ca^{2+} stores, the effects of ACh on the NSC current are blunted (65, 67). Use of heparin to block IP_3 release of Ca^{2+} from intracellular stores markedly reduces the rise in intracellular Ca^{2+} evoked by ACh (66). As a rise in intracellular Ca^{2+} is not sufficient to activate the NSC in most preparations, it appears that either ACh binding to its receptor or activation of a G protein is necessary before Ca^{2+} can activate the NSC.

ACH AND G PROTEINS The effects of muscarinic stimulation have been extensively studied in gastrointestinal smooth muscle. At a channel level, muscarinic stimulation has been shown to modulate Ca^{2+} channels (see above), K^+ channels (see below), and NSC channels. The effects of ACh on a NSC current were first reported (59) in rabbit jejunal myocytes. The effects of ACh on the NSC current appear to be mediated by pertussis toxin-sensitive G proteins. Inoue & Isenberg in 1990 (64) examined the effect of G-protein activation on guinea pig ileal longitudinal myocytes. A K^+-free pipette solution was used to isolate the NSC current and cells were clamped at -50 mV. GTP-γS (100 μM) activated the inward current, and ACh (300 μM), applied after GTP-γS, was without effect. Also, GDP-βS, an inhibitor of G-protein activation, inhibited the effects of ACh on the NSC current. These results suggest that the effects of ACh are mediated by a G protein. The NSC current was blocked by pertussis toxin. Similar results were obtained by Komori et al (68), who also showed that histamine activated the NSC current by the same pertussis toxin-sensitive G-protein mechanism. Activation of a NSC current by ACh has now been reported for rabbit jejunal myocytes (59); guinea pig myocytes (69); canine pyloric, jejunal, and colonic myocytes (24, 55, 67, 70); *Bufo marinus* myocytes; opossum esophageal myocytes (43); and mouse anococcygeus myocytes (72). The carbachol-activated NSC current in guinea pig myocytes was inhibited by activation of PKC (73).

pH The ACh-activated NSC current in longitudinal myocytes from guinea pig ileum is pH sensitive (61). Acidification of the extracellular solution to a pH

of 6.0 doubled the ACh-activated NSC current, and alkalization to a pH of 7.75 halved the ACh-activated NSC current. The effects of pH were secondary to an increase in H^+ conductance because no effect of pH change was seen in the absence of ACh or to changes in intracellular $[Ca^{2+}]$; the same effects were observed with 20 mM BAPTA in the pipette. These results suggest a direct modulation of the NSC current by H^+. The effects of pH on the ACh-activated NSC current could be described with a Hill-type equation with an apparent pK_a of 7.4 and a Hill coefficient of ≈ 1. Acidification shifted the peak of the inward current-voltage relationship to the left, and steady-state activation shifted from a half-maximal activation voltage of -58 mV at pH 7.4 to -68 mV at a pH of 7.0.

POTASSIUM CHANNELS

Introduction

Most excitable cells, including gastrointestinal smooth muscle cells, express several types of K^+ channels, with the most common being delayed rectifier and Ca^{2+}-activated types. Other K^+ channel types include A-type, inward rectifier, and ATP-sensitive channels. Given the large diversity of K^+ channels, even within their subgroups, each type of K^+ channel is discussed separately.

DELAYED RECTIFIER K^+ CHANNELS

Introduction

Delayed rectifier K^+ channels have been found in all smooth muscles studied. The term delayed rectifier was coined to describe a K^+ channel that opens after a brief delay in response to a depolarization, and it is now loosely applied to K^+ channels that are not Ca^{2+} activated, do not inactivate rapidly, and do not show inward rectification regardless of whether there is a delay in activation. Many delayed rectifiers K^+ channels are open at the resting membrane potential of gastrointestinal smooth muscle and hence contribute to setting the membrane potential. Opening of a delayed rectifier K^+ channel would pull the membrane potential toward E_K, usually ≈ -80 mV. The membrane potential of gastrointestinal smooth muscle cells is usually positive to E_K. The membrane potential is therefore set by the combination of K^+ channels and other channels open at the resting membrane potential, such as chloride and NSC channels.

Channel Classification

The first K^+ channel to be sequenced was the A-type K^+ channel from *Drosophila*. The *shaker* locus on the X chromosome encodes for a gene whose cDNA clones resulted in expression of functional A-type K^+ channels, the

shaker K^+ channel. Probes from the *shaker* locus were then used to identify related K^+ channels, called *shal*, *shab*, and *shaw*. In the progression from *shaker* to *shaw*, inactivation lengthens and the channels become more like delayed rectifier channels. A myriad of delayed rectifier K^+ channels have now been cloned, as well as subunits that do not express a functional K^+ channel on their own but modify the behavior of other K^+ channels. Delayed rectifier K^+ channels are now classified by their gene family into nine families of α-subunits, K_V1–K_V9, as well as K_VLQT1. Additional families are likely to be expressed in the future. K_V1–K_V4 and K_V7 are electrically active; K_V5, 6, 8, and 9 are electrically silent but when combined with the electrically active subunits alter their electrical behavior. Also in the current classification are β-subunits $K_V\beta$-1, $K_V\beta$-2, and $K_V\beta$-4, which are cytoplasmic subunits with little homology with α-subunits but modify their behavior. The current classification is maintained at http://qlink.queensu.ca/~4jch3/Kchseq.htm. In gastrointestinal smooth muscle, $cK_V1.2$ and $cK_V1.5$ have been cloned from canine gastrointestinal myocytes (74, 75). $cK_V1.2$ had a single channel conductance of 14 pS (symmetrical 140 mM K^+). The macroscopic current resulting from expression of $cK_V1.2$ increased with voltages positive to −40 mV. The current had a fast activation time (time to half-peak = 7.4 ms), exhibited slow and incomplete inactivation, was blocked by 4-AP (IC_{50} of 75 μM), and was resistant to TEA (11% block by 10 mM). Based on its electrophysiological and pharmacological characteristics, $cK_V1.2$ was thought to be most closely similar to $I_{dK(f)}$ in the native colonic circular smooth muscle whole-cell current (see below). The single channel conductance (symmetrical 140 mM K^+) of $cK_V1.5$ was 10 pS. Like $cK_V1.2$, the macroscopic current resulting from expression of $cK_V1.5$ increased with voltages positive to −40 mV. The current had a fast activation time (time to half-peak = 5.5 ms), exhibited slow and incomplete inactivation, was blocked by 4-AP (IC_{50} of 211 μM), and was resistant to TEA (less than a 10% block by10 mM). Based on the electrophysiological and pharmacological characteristics, $cK_V1.5$ closely resembled $cK_V1.2$, suggesting that $I_{dK(f)}$ is made up of more than one channel type or that the native current is made up of $K_V1.2$ and $cK_V1.5$ heterotetramers (76).

Channel Structure

Delayed rectifier K^+ channels are made up of at least four identical α-subunits. Each subunit has six membrane spanning regions. α-subunits need to be expressed together or with β-subunits to approximate native delayed rectifier current.

Single Channel Conductance

Several different single channel conductances have been reported for different delayed rectifier channels. In gastric myocytes from *Rana pipiens* and *Bufo*

marinus, two channels were identified with conductances of 15 and 40 pS. The 40 pS channel was blocked by quinidine (100 μM) and the 15 pS channel by TEA, but not by quinidine (77). Also in *Bufo marinus* gastric myocytes, a 20 pS channel was characterized that was responsive to stretch and fatty acids, suggesting it was a stretch-activated K^+ channel (78).

In rabbit longitudinal jejunal myocytes, a 50 pS (internal $K^+ = 5.9$ mM, external $K^+ = 126$ mM) delayed rectifier K^+ channel was identified. The channel was insensitive to Ca^{2+} and blocked by cytoplasmic TEA (5–10 mM). Mean open time was 16.4 ms and mean closed time within a burst was 6.4 ms. As activation times were slow and open probability began to increase around −45 mV, it is likely that this channel type does not contribute significantly to the membrane potential, and action potentials can occur without activation of this channel type (79). In the small intestine, a 186 pS K^+ channel was identified in neonatal mouse myocytes from an explant. Channel activity was not affected by buffering Ca^{2+} with EGTA, was not blocked by intracellular ATP, and single channel gating was inhibited by 4-AP (100 μM). Also, the ensemble current turned on after a brief delay, leading the authors to suggest that it was a delayed rectifier K^+ channel.

In colonic circular myocytes, 19- and 90-pS channels (symmetrical 140 mM K^+) were identified that had delayed-rectifier properties (80). The 19-pS channel was inhibited by 4-AP (10 mM), TEA (10 mM), and was not Ca^{2+} dependent. Most channel openings were clustered at the beginning of depolarizing pulses, suggesting substantial inactivation. Inactivation was, however, never complete, with channel openings still resolvable at steady state depolarized voltages. The 90-pS channel was variably sensitive to 4-AP, TEA, and Ca^{2+}, suggesting a class rather than a single channel type. Protein kinase A activated both channel types, but at −60 mV (a physiological membrane potential), only the 19-pS channel was activated. VIP (100 nM) activated the 19-pS channel increasing open probability, mean open time, and mean burst duration (18). Also, in canine colonic myocytes there is 4-pS K^+ activated by nitric oxide but not by voltage, suggesting it may not be a delayed rectifier K^+ channel (82).

Whole-Cell Current Kinetics and Regulation

Whole-cell delayed rectifier current kinetics were studied in human and canine jejunal circular smooth muscle cells at 24°C. In both, activation was not voltage dependent (83, 84). Activation time was 90 ± 25 ms and 80 ± 25 ms, respectively. Deactivation was weakly voltage dependent, with a τ of 10 ± 3 ms at −140 mV and 40 ± 32 ms at −60 mV for canine myocytes and a τ of 13 ± 4 ms at −80 mV and 22 ± 11 ms at −40 mV for human myocytes. The current was not dependent on holding voltage (10% decrease in current at 0 mV compared with −60 mV). Less than 15% inactivation was noted over 2 s. The open probability of the delayed rectifier K^+ channels contributing to the whole-cell

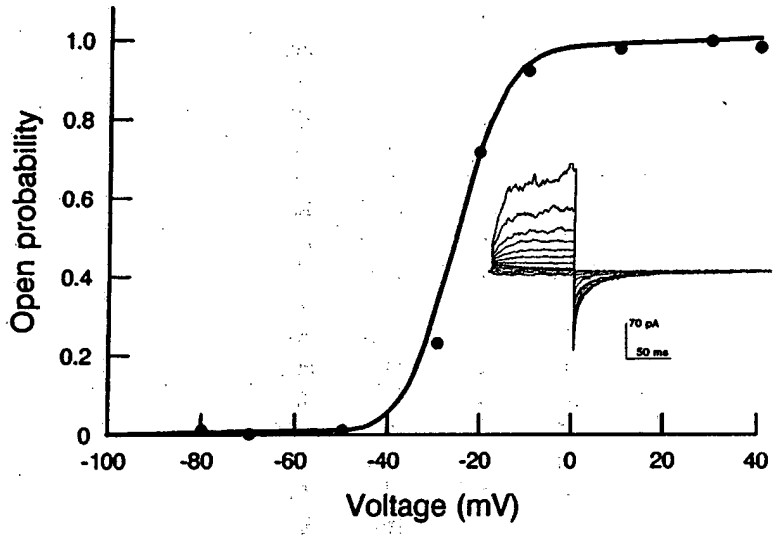

Figure 2 Open probability of the delayed rectifier channels carrying the whole-cell current. Normalized tail currents were obtained from tail currents (*inset*) of canine jejunal circular smooth muscle cells with 150 KCl in the bath. Open probability began to increase at −60 mV (83).

current was determined from tail current protocols generated in 150 mM KCl (Figure 2). The open probability of the canine delayed rectifier current began to increase at about −60 mV and reached unitary open probability at 0 mV. The human delayed rectifier current began to increase at about −70 mV and reached unitary open probability at 10 mV. Therefore the currents would be expected to contribute to the membrane potential of human and canine jejunal circular smooth muscle myocytes. In human myocytes, quinidine (100 μM) blocked a substantial portion of the whole-cell current and depolarized the membrane potential to near 0 mV, again suggesting that the whole-cell delayed rectifier K^+ current is a major determinant of the membrane potential.

The whole-cell delayed rectifier current in canine colonic circular myocytes included three components discernible by their TEA and 4-AP sensitivities (85). Time to half-maximal activation of the control current was 26 ± 4 ms. TEA (10 mM) blocked 50% of the outward current and the time to half-maximal activation of the residual current was 17 ± 2 ms. In contrast, 4-AP blocked 35% of the outward current and the time to half-maximal activation of the residual current was 40 ± 8 ms, suggesting that 4-AP blocked a fast activation component of the whole-cell delayed rectifier current ($I_{dK(f)}$) and TEA a slow-activating component ($I_{dK(s)}$). 4-AP block was use dependent but not frequency or voltage dependent, suggesting that the slowing of activation times by 4-AP

was not the result of a state-dependent block but rather block of a component of the whole-cell current. The third component of the whole-cell delayed rectifier current was revealed by block with TEA (10 mM) in the pipette. In the absence of TEA, the current inactivated by only 10% during a 20 s pulse to −50 mV and in the presence of intracellular TEA by 80%, suggesting the presence of a low inactivation threshold current ($I_{dK(n)}$). This current was insensitive to 4-AP and sensitive to external TEA.

PHOSPHORYLATION The delayed rectifier current in guinea pig taenia coli myocytes was activated by phosphorylation (86). The membrane permeant form of cAMP, dibutyryl cAMP, increased the K^+ current, and H-8 (a protein kinase A inhibitor) inhibited the effect of dibutyryl cAMP. Calyculin A also increased outward K^+ current, suggesting that the delayed rectifier type current is regulated by protein kinase A-mediated phosphorylation. At the single channel level, the 20 and 90 pS delayed rectifier channels identified in colonic myocytes were also activated by protein kinase A. Application of the catalytic subunit of protein kinase A (10 U/ml) to inside-out patches activated both channels (80).

VIP In canine colon circular smooth muscle cells, VIP increased a charybdotoxin (ChTX)-insensitive, voltage-dependent K^+ current (81). VIP is known to relax gastrointestinal smooth muscle and is an inhibitory neurotransmitter found in enteric nerves. At a single channel level, VIP activated a 20 pS 4-AP-sensitive K^+ channel, suggesting that the delayed rectifier 20 pS channel may underlie the effects of VIP in colonic smooth muscle.

FENAMATES AND FLUOXETINE The delayed rectifier current characterized (83, 84) in human and canine jejunal circular smooth muscle cells is sensitive to fenamates, which are nonsteroidal antiinflammatory drugs in clinical use. Use of these drugs is associated with gastrointestinal motility disturbances in up to a third of patients. Flufenamic acid and mefenamic acid, both fenamates, activated the delayed rectifier current at concentrations equivalent to therapeutic blood levels. Similarly, fluoxetine, a commonly prescribed antidepressant (Prozac®), and its metabolite norfluoxetine had profound effects on the delayed rectifier current in both human and canine jejunal circular smooth muscle cells (129). Fluoxetine inhibited the current at concentrations equivalent to therapeutic blood levels and at higher concentrations stimulated a Ca^{2+}-activated ChTX sensitive current by increasing intracellular Ca^{2+} levels.

LOW-MOLECULAR-WEIGHT OXIDES Low-molecular-weight oxides regulate colonic and jejunal myocyte delayed rectifier current. In colonic circular myocytes, nitric oxide (NO) and sodium nitroprusside activate an 80 pS delayed rectifier channel (82). As the effects of nitric oxide are often mediated through

activation of cGMP-dependent protein kinase, the effects of the membrane-permeant form of cGMP, dibutyryl cGMP, were tested on the K^+ channel. Dibutyryl cGMP increased P_o in cell-attached patches. However, in inside-out patches, NO still increased the open probability of the 80 pS channel, raising the possibility of a direct action on the channel. As patches may contain cytoplasmic components, an indirect effect of NO cannot be ruled out. NO also activated two other K^+ channels, a voltage-insensitive ≈4-pS channel and a Ca^{2+}-activated 250-pS channel. NO may therefore contribute to several phases of the inhibitory response in the gastrointestinal tract. At the resting membrane potential, the 4 pS and 80 pS channels may be modulated by NO released from enteric nerves, while under conditions where the muscle is depolarized or intracellular Ca^{2+} is raised, such as at the peak of the slow wave, the 250 pS Ca^{2+}-activated K^+ channel may be activated.

Carbon monoxide (CO) is another low-molecular-oxide putative messenger in the gastrointestinal tract. Exogenous CO activates the delayed rectifier current in both human and canine jejunal circular smooth muscle cells, resulting in a cyclic oscillation in the membrane potential (87, 88). The enzyme that produces CO, heme oxygenase, was found in a subpopulation of enteric neurons and interstitial cells of Cajal (89), raising the possibility that CO released from nerves and interstitial cells modulates smooth muscle delayed rectifier activity and membrane potential.

A-TYPE K^+ CHANNELS

Introduction

A fast-inactivating K^+ current was first described in molluscan neurons (90). Characteristic properties of this current include a steep holding voltage dependence, with near-complete inactivation at −50 mV, fast kinetics of activation and inactivation, and high sensitivity to block by 4-AP. In neurons, A-type K^+ currents are mostly inactivated at the resting membrane potential and activated during and after hyperpolarization to slow the rate of pacemaker depolarization, modulating the action potential frequency. The role of an A-type K^+ current in gastrointestinal smooth muscle is less clear. The A-type K^+ current in guinea pig colonic myocytes and esophageal myocytes is not completely inactivated at the resting membrane potential and may therefore contribute to the membrane potential, modulating the generation of action potentials by opposing the inward Ca^{2+} current (91, 92).

Channel Classification

A-type K^+ channels are now classified by their gene family into the K_V subfamilies, with most falling into the K_V1 subfamily. K^+ channels with A-type characteristics have not yet been reported in gastrointestinal smooth muscle.

Channel Structure

Recently, Doyle et al (93) used X-ray crystallography to determine the structure of KcsA, a K^+ channel from *Streptomyces lividans*. The pore region of KcsA is nearly identical to the *Drosophila shaker* K^+ channel as well as to other vertebrate voltage-gated K^+ channels. The channel is a tetramer with four identical units around a central pore. Each subunit had two transmembrane α-helices and the two α-helices are connected by the pore region (roughly 30 amino acids). One α-helix of each subunit faces outward and one faces the central pore. The selectivity filter contains two ions 7.5 Å apart and the attraction force between the selectivity filter is perfectly balanced, with repulsive forces between the two K^+ ions, allowing the channel to conduct with a high throughput and with high selectivity (93).

Single Channel Conductance

The only report of single channel recording from ion channels thought to carry an A-type K^+ current in gastrointestinal smooth muscle is from Vogalis & Lang (94). A K^+ channel with a conductance of 12–13 pS was recorded from on-cell patches of guinea pig colonic myocytes (K^+ in pipette = 130 mM, in bath 6 mM). The channel was blocked by 4-AP (5 mM) but not by TEA (5 mM), and inactivated within 50 ms. The channel was seen only following step depolarizations from a hyperpolarized voltage. Its kinetic features closely resembled the whole-cell A-type current recorded from guinea pig colonic myocytes, suggesting it carried the whole-cell current (91).

Although the cloned K^+ channels from canine colon, $cK_V1.2$ and $cK_V1.5$, fall in the *shaker* family of K^+ channels, the expressed channels do not exhibit rapid inactivation and are not considered A-type K^+ channels.

Whole-Cell Current Kinetics and Regulation

The first description of an A-type K^+ current in gastrointestinal myocytes was from rat ileal myocytes (95). Two major currents were found in newborn and adult rat ileal myocytes: a delayed rectifier type current, and a fast-inactivating current labeled I_{fo}. At a holding voltage of −80 mV, step depolarizations to −40 mV revealed an outward current that peaked within 10 ms and inactivated rapidly. Activation was voltage dependent, with a time to peak of 10.8 ± 0.9 ms at −30 mV and 6.7 ± 1.2 ms at 20 mV. I_{fo} decay was well fit with a single exponential, with a time constant of 17–33 ms. Recovery from inactivation was also well fit with a single exponential. Recovery from inactivation was voltage dependent, with a time constant of 28 ms at −120 mV and 90 ms at −80 mV. Current kinetics were dependent on external $[Ca^{2+}]$, with an increase in peak current and a 16-mV shift in the hyperpolarized direction when Ca^{2+} was removed from the bath. I_{fo} was weakly TEA sensitive and 4-AP sensitive. 4-AP (1 mM) blocked 40% of the outward current.

In rabbit esophageal muscularis mucosae myocytes, a transient outward K^+ current was recorded using the nystatin perforated patch technique (96). The transient K^+ inactivated over a period of about 50 ms. However, the current was Ca^{2+} dependent and blocked by TEA (5 mM), suggesting it was not an A-type K^+ current.

In esophageal circular myocytes (92) and in guinea pig colonic myocytes (91), overlap of steady-state activation and inactivation curves reveals a window current between −60 and −30 mV, suggesting that the A-type K^+ current can contribute to electrophysiological events at voltages approximating the membrane potential. A-type K^+ currents have also been reported in guinea pig ileum, human jejunum, and rat distal colonic myocytes (97–99).

In guinea pig colonic myocytes the A-type current is blocked by arachidonic acid (1–10 μM). Block by arachidonic acid was not dependent on protein kinases, G proteins, or indomethacin. Arachidonic acid was the most potent blocker of all fatty acids tested. As arachidonic acid had the most unsaturated double bonds (4) of the fatty acids tested, it was suggested that arachidonic acid blocked A-type K^+ channels through a stereospecific mechanism (100).

Ca^{2+}-ACTIVATED K^+ CHANNELS

Introduction

Ca^{2+}-activated K^+ channels are a diverse group of K^+ channels that share a common property: an increase in open probability as internal Ca^{2+} rises. Three subgroups of Ca^{2+}-activated K^+ channels can be identified based on their single channel conductance: large conductance (BK), intermediate conductance (IK_{Ca}), and small conductance (SK_{Ca}).

BK may contribute to repolarization after an excitable event. ChTX has little effect on basal electrical or mechanical activity of gastrointestinal smooth muscle in most preparations, which suggests that, at rest, BK does not contribute significantly to mechanical activity or membrane potential. However, in a study of the effects of ChTX on guinea pig ileum, longitudinal muscle ChTX induced spike-like depolarizations, increased spontaneous activity, and inhibited nerve stimulation-evoked membrane hyperpolarization, which suggests BK in this preparation was constitutively active (101). Also, when gastrointestinal smooth muscle strips are activated by ACh, ChTX increases electrical slow-wave duration and amplitude and increases contractile activity, suggesting that BK plays a role in the control of excitability of gastrointestinal smooth muscle to counter large unopposed effects of excitatory neurotransmitters.

IK_{Ca} and SK_{Ca} may play central roles in the generation of inhibitory junction potentials (IJPs). IJPs are membrane hyperpolarizations accompanied

by muscle relaxation evoked by inhibitory neurotransmitter release from enteric nerves. Abnormalities in IJPs have been implicated in constipation and other gastrointestinal motility disorders. Recent evidence suggests that ATP-activated IK_{Ca} and SK_{Ca} are present in gastrointestinal smooth muscle cells and, based on their pharmacology, generate IJPs (102, 103).

BK channels have a conductance of ≈80–250 pS, are activated by micromolar concentrations of Ca^{2+}, regulated by voltage, and blocked by ChTX. IK_{Ca} have a conductance of ≈40 pS, have higher sensitivity to Ca^{2+} than BK, are voltage insensitive, and are blocked by apamin and millimolar concentrations of TEA. SK_{Ca} have a conductance of 5–10 pS, have a higher sensitivity to internal Ca^{2+} than BK, are voltage insensitive, and are blocked by apamin but are resistant to millimolar TEA.

Channel Structure

BK is made up of α- and β-subunits. Both have been cloned in canine colonic smooth muscle (cslo-α and cslo-β) (104). The α-subunit has a M_r of 62,000 and when expressed alone can conduct K^+ and is voltage and Ca^{2+} sensitive. The β-subunit has a M_r of 22,000, and both NH_2 and COOH terminals are cytoplasmic. The β-subunit has a site for phosphorylation by protein kinase A. Expression of the β-subunit alone does not lead to the appearance of a functional ion channel, but expression of both subunits together shifts the voltage dependence of activation by ≈50 mV as well as increases Ca^{2+} sensitivity.

Single Channel Conductance

BK channels in gastrointestinal smooth muscle have conductances varying from 100 to 220 pS (reviewed in 105). Larger and smaller conductances have been reported in other cells. The slope conductance of the cloned BK channel composed of the α-subunit alone or of the α- and β-subunits together was 207–215 pS at 1 μM free Ca^{2+}. TEA (100–500 μM) produced a characteristic flickery block and an apparent decrease in unitary current. ChTX and iberiotoxin completely blocked channel openings, features characteristic of BK.

IK_{Ca} have been studied in murine ileal and colonic myocytes and SK_{Ca} in murine ileal myocytes. In the ileum, IK_{Ca} and SK_{Ca} were identified in cell-attached patches (bath K^+ = 2.5 mM, pipette K^+ 2.5 mM), with an external Ca^{2+} concentration of 150 nM. The conductance of the IK_{Ca} was 39 pS and of the SK_{Ca} estimated at 10 pS. IK_{Ca} and SK_{Ca} were activated by the P2Y agonist 2-MeSATP (20–50 μM). SK_{Ca} was not blocked by 2 mM TEA in the pipette and IK_{Ca} was only partly blocked by apamin. In the colon, SK_{Ca} were recorded from inside-out patches. In symmetrical K^+ solutions (140 mM), the channel conductance at a bath [Ca^{2+}] 100 nM was 5 pS. The P_o was increased by caffeine, ATP, 2-MeSATP (10 μM), and UTP (1 mM). In inside-out patches, at 10 nM

external Ca^{2+} little activity of SK_{Ca} was noted. Open probability increased as bath Ca^{2+} concentration increased with a 50% effective concentration of Ca^{2+} of 500 nM. The channel showed no voltage or time dependence.

Whole-Cell Current Kinetics and Regulation of BK

A signature of the whole-cell current produced by BK channels is noise. As the single channel conductance is large, the whole-cell current often appears to be noisy. Another feature of BK is fast deactivation on stepping to hyperpolarized voltages, giving rise to fast tail currents, with τ of a few ms.

CALCIUM DEPENDENCE BK channel activity is dependent on intracellular Ca^{2+} concentration. In a study in excised patches from canine gastric myocytes (106), a change in Ca^{2+} concentration from 100 nM to 1 μM at the inner surface of the patch resulted in an a $\sim$100 mV negative shift in the voltage for half-maximal activation. At a holding voltage of 0 mV, activation had a Hill coefficient of 3.4, suggesting Ca^{2+} cooperativity in the activation of the channel. In similar experiments carried out in canine colonic myocytes (107), a change of the Ca^{2+} concentration from 100 nM to 1 μM resulted in a 130-mV shift in the voltage for half-maximal activation. At +50 mV, activation had a Hill coefficient of 5.3, again suggesting high cooperativity in the activation of the colonic channel by Ca^{2+}.

Substance P (SP) activates BK in rabbit longitudinal colonic myocytes through an increase in intracellular [Ca^{2+}] (108). SP (10^{-12} M) activated a BK channel with a conductance of $\approx$165 pS (pipette = 126 mM K^+) in on-cell patches and 198 pS in inside-out patches (pipette and bath K^+ = 126 mM). The P_o increased tenfold when Ca^{2+} was increased from 50 nM to 5 μM, and TEA (10 mM) blocked the channel. Activation of BK by SP was dependent on extracellular Ca^{2+} as nifedipine decreased the P_o.

Nitric oxide (NO) activates Ca^{2+}-dependent K^+ currents in esophageal circular myocytes as well as colonic myocytes. Sin-1 and sodium nitroprusside, NO donors, activated a whole-cell K^+ current, and activation was blocked by cyclopiazonic acid, a sarcoplasmic reticulum (SR) Ca^{2+}-pump inhibitor. This suggests that activation depends on release of Ca^{2+} from the SR (109). NO activates a 250 pS Ca^{2+}-activated channel in on-cell patches from canine colon circular myocytes (82).

VOLTAGE DEPENDENCE Activation of BK by voltage is intimately linked to Ca^{2+}. Ca^{2+} may alter the voltage sensor, which alters the binding of Ca^{2+} to BK. BK is strongly voltage dependent. At constant intracellular [Ca^{2+}], BK is activated by depolarization and closed by hyperpolarization. In experiments carried out on coexpressed α- and β-subunits from canine colonic myocytes in

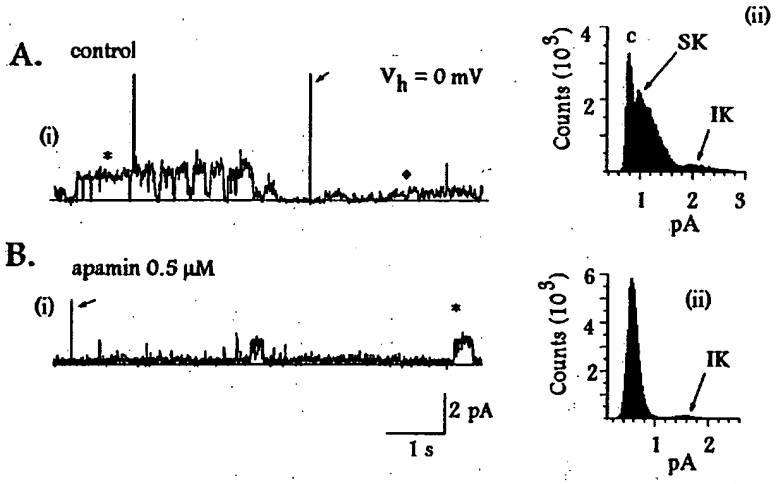

Figure 3 BK (*arrow*), IK_{Ca} (*) and SK_{Ca} (◆) channels recorded from a cell-attached patch from mouse ileum (*panel A*). Apamin (0.5 μM, *panel B*) blocked SK_{Ca} but not BK or IK_{Ca} (102).

inside-out patches, with a bath $[Ca^{2+}]$ of 1 μM, the macroscopic current began to activate at 0 mV and reached full open probability at ≈100 mV (104).

Whole-Cell Kinetics and Regulation of IK_{Ca} and SK_{Ca}

At a single channel level, IK_{Ca} and SK_{Ca} in murine ileal myocytes were found to be activated by ATP and blocked by apamin (Figure 3). These features were used to identify the whole-cell current correlate (102). The whole-cell current, recorded using the amphotericin perforated patch technique, was blocked by apamin and activated by ATP. It had an initial transient outward component and a sustained outward component. Apamin (0.5 μM) blocked about 70% of the sustained current at +50 mV. ATP increased both the initial transient outward component and the sustained outward component of the whole-cell current and apamin blocked the increase in current evoked by ATP. The current-voltage relationship revealed initial outward currents at voltages positive to −50 mV. The current was dependent on intracellular $[Ca^{2+}]$. In traditional whole-cell experiments, the initial transient outward current was not seen, and at 150 nM (but not at 85 nM internal Ca^{2+}) an apamin-sensitive current was present. Similar experiments were carried out in murine colonic myocytes (103). ATP (1 mM) activated a K^+ conductance with initial activation seen at about −80 mV and little or no voltage dependence. Similarly, the putative P2Y receptor agonist 2-MeSATP (100 μM) and UDP (1 mM) activated a whole-cell

current with characteristics similar to that activated by ATP. Apamin (300 nM) blocked about 44% of the current stimulated by ATP.

ATP-SENSITIVE AND INWARDLY RECTIFYING POTASSIUM CHANNELS

Introduction

In this family there are two main types of K^+ channels: ATP-sensitive (K_{ATP}) and inward rectifier (K_{IR}). K_{IR} channels have not been found in gastrointestinal smooth muscle, and it is unclear whether K_{ATP} channels are expressed in gastrointestinal smooth muscle cells. However, as K_{ATP} channel modulators do have effects on ionic currents in these cells, this review focuses on K_{ATP} channels. K_{ATP} channels show weak inward rectification and are sensitive to ATP. A decrease in intracellular ATP, such as would occur with metabolic inhibition or hypoxia, activates K_{ATP} channels and an increase in ATP closes them. K_{ATP} channels link K^+ flux across a cell membrane to the metabolic state of the cells. Characteristic features of K_{ATP} channels are that they are inhibited by sulfonylureas such as glibenclamide and tolbutamide and activated by cromakalim, minoxidil, pinacidil, and diazoxide (reviewed in 110).

Channel Classification

K_{ATP} and K_{IR} channels have similar structures and are classified into $K_{IR}1$ to $K_{IR}6$ subfamilies. Inward rectifier K^+ channels fall into $K_{IR}1$ to $K_{IR}3$ (K_{IR}). $K_{IR}4$ are ATP-dependent K^+ channels and $K_{IR}6$ ATP-sensitive K^+ channels (K_{ATP}).

Channel Structure

Both K_{ATP} and K_{IR} channels share a similar structure with two transmembrane domains in each subunit. It appears that, like the *shaker* K_V channel, four subunits aggregate to form a functional channel. K_{ATP} channels are made up of $K_{IR}6$ subfamily subunits that associate with the sulfonylurea receptor. The sulfonylurea receptor belongs to the ATP-binding cassette ATPase superfamily.

Single Channel Conductance

No single channel studies on K_{ATP} channels in the gastrointestinal tract have been reported. In vascular smooth muscle, unitary conductance can be divided into two groups, a small conductance family (15–50 pS) and a large conductance family (100–258 pS) (reviewed in 110).

Whole-Cell Current Kinetics and Regulation

Some of the first evidence for the presence of K_{ATP} channels in gastrointestinal smooth muscle came from a study of the effects of sulfonylureas and

glibenclamide on a guinea pig longitudinal smooth muscle/myenteric plexus preparation (111). Cromakalim and its isomer lemakalim activate a whole-cell K^+ current in canine colonic myocytes (112). Activation was inhibited by the sulfonylurea glyburide. However, a subsequent study showed that the predominant effects of cromakalim and lemakalim were on a Ca^{2+}-activated K^+ channel, questioning the presence of K_{ATP} in canine colonic myocytes (113).

In rabbit esophageal muscularis mucosa myocytes, lemakalim (10 μM) activates a whole-cell current that is ATP sensitive and Ca^{2+} independent (114). The current-voltage relationship of this current showed a substantial inward current compatible with K_{ATP}. Carbachol, via the M_3 receptor and PMA (membrane permeant PKC activator) inhibited the current, an effect attenuated by tyrosine kinase inhibitors. These results suggest that K_{ATP} is present in rabbit esophageal muscularis mucosa myocytes.

SODIUM CHANNELS

Introduction

The presence of Na^+ channels in gastrointestinal smooth muscle is controversial. The main function of Na^+ channels in excitable cells is to generate the rapid upstroke of the action potential, resulting in a rapid depolarization. Na^+ channels also play a role in the generation of pacemaker and subthreshold potentials. In gastrointestinal smooth muscle, the upstroke of the action potential is predominantly due to Ca^{2+} entry and therefore Na^+ channels are not essential in the generation of the action potential. However, some data suggest that Na^+ channels can be found in gastrointestinal smooth muscle, although their physiological role is presently unclear.

Channel Classification

Excitable-membrane sodium channels can be classified based on their sensitivity to tetrodotoxin (TTX), the paralytic poison derived from fish of the order Tetraodontiformes. TTX selectively blocks Na^+ channels with varying kinetics, defining two main types of Na^+: fast TTX-sensitive and slow TTX-insensitive.

Channel Structure

The basic structure of a Na^+ channel is of four homologous protein domains thought to be arranged around a central pore.

Single Channel Conductance

Single channel Na^+ currents have not been measured from gastrointestinal smooth muscle.

Whole-Cell Current Kinetics and Regulation

Ohya et al reported that, in rabbit ileal longitudinal smooth muscle cells, removal of extracellular Ca^{2+} unmasked a Na^+ current at depolarized potentials. The current was revealed when extracellular Ca^{2+} was reduced to 100 nM. However, the current was TTX insensitive and blocked by Ca^{2+} channel blockers, suggesting that it was due to Na^+ influx through Ca^{2+} channels rather than through a Na^+ channel (115). Inoue et al (54) studied single channel activity evoked by ACh in single guinea pig ileum smooth muscle cells. In their experiments the pipette solution contained Mg^{2+} (5 mM) and no Ca^{2+}. ACh activated atropine-sensitive channels with a single channel conductance of 20–25 pS and a permeability ratio of $Na^+ : K^+$ of 1.0 : 0.3–0.4. The permeability ratio suggests a NSC channel, although removal of external Na^+ resulted in loss of channel activity over a wide range of membrane potential (−30 to −80 mV), suggesting that in physiological ionic environments the channel was predominantly Na^+ selective. Although the channel appears to function as a Na^+ channel, it is still considered a NSC channel (54).

A TTX-sensitive Na^+ current was recorded from rat ileum single smooth muscle cells in nominally Ca^{2+}-free solution (32) and from rat fundus smooth muscle cells (116, 117). The strongest evidence for expression of Na^+ channels in gastrointestinal smooth muscle was provided by Xiong et al (118). Single adult rat and human colonic smooth muscle cells were patch clamped in the presence of 4-AP in the bath solution and Cs^{2+} in the pipette solution to block K^+ currents. Cells were held at −100 mV and step depolarized. At voltages positive to −40 mV two components of an inward current were observed: The slower component was was blocked by nifedipine and Ni^{2+}, suggesting it was carried by Ca^{2+} channels; the other fast component was blocked by TTX, unaffected by 0 mM external Ca^{2+}, nifedipine or Ni^{2+}, and disappeared when external Na^+ was substituted with choline, suggesting that it was a Na^+ current (Figure 4). The Na^+ current had half-inactivations of −74.5 and −69.5 mV, and slope factors of 12.2 and 7.6 mV in rat and human cells, respectively. Half-activation was −36.1 and −21.8 mV, with slope factors of 5.8 and 7.6 mV in rat and human cells, respectively. As the activation and steady-state inactivation curves overlapped between −60 and −30 mV, a substantial window current was present. The incidence of the Na^+ current was higher in the proximal colon (16 of 22 human cells) when compared with the distal colon (7 of 32 cells). The uneven distribution of Na^+ current raises an interesting question: Were the Na^+ currents recorded from cells derived from colonic muscularis propria or from contaminant cells? The most likely source of contaminant cell is smooth muscle cells from the muscularis mucosae and interstitial cells of Cajal (ICC). Contamination is unlikely because the muscularis mucosae was removed in

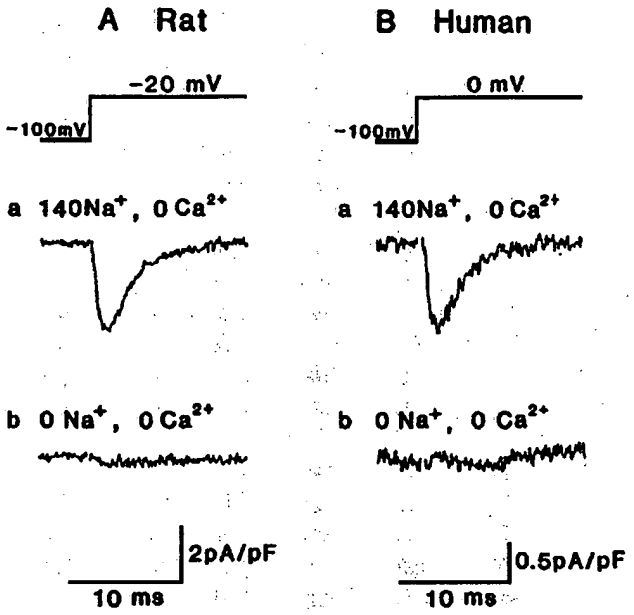

Figure 4 Na^+ currents recorded from rat (*A*) and human (*B*) colonic smooth muscle cells. Removal of Na^+ ions from the bath resulted in loss of the Na^+ current (118).

these experiments and the interstitial cells of Cajal occupy less than 3% of the smooth muscle volume.

IONIC CURRENTS IN INTERSTITIAL CELLS OF CAJAL

Introduction

It has recently been established that ICC generate the electrical slow wave recorded from muscle cells in muscle strip preparations (120, 121). ICC express c-Kit, a tyrosine receptor kinase, that is required for the development of a subpopulation of ICC. Mice partly lacking c-Kit do not have ICC associated with the myenteric plexus in the small intestine, unlike normal controls. No slow wave can be recorded from the small intestine of these mice and intestinal peristalsis is markedly altered and inefficient (120, 121). ICC may serve not only to generate the slow wave but also to amplify neuronal input to gastrointestinal smooth muscle cells. Loss of ICC has been implicated in human motility disorders such as hypertrophic infantile stenosis (122), Hirshsprung's

disease (123), severe constipation, and intestinal pseudo-obstruction (124) and in inflammatory bowel disease (125). It is therefore of great interest to determine the ionic conductances of ICC as they appear to be central to the regulation of gastrointestinal motility. As relatively little is known about ionic currents in ICC, they are grouped together.

Ion Channels

Initial patch clamp recording of ICC were performed in dog colon (126). ICC were isolated from the submucosal surface of the circular muscle layer, known to be a pacemaker region of the colon. Ca^{2+}-activated K^+ channels with a similar conductance (218 ± 25 pS) to colonic circular smooth muscle myocytes were present in on-cell patches. The voltage- and Ca^{2+}-dependence of Ca^{2+}-activated K^+ channels in ICC were also similar to colonic myocytes. Ca^{2+} currents were also studied in this preparation. Inward Ca^{2+} current activated within 5 ms and was blocked by nifedipine (1 μM). The current-voltage relationships of the whole-cell inward current revealed initial activation at -60 mV, with a peak at 0 mV, although initial activation was more negative in the figure shown (-70 mV). Steady-state activation and inactivation curves revealed a window current, suggesting a sustained Ca^{2+} influx.

The ionic current in canine colonic ICC was further studied and compared with canine colonic myocytes using both standard whole-cell recording techniques and the perforated patch clamp technique. Outward current in standard whole-cell recordings (1 mM EGTA in pipette) activated to peak within 14 ms and inactivated by 60% within 500 ms. The outward current was resistant to 4-AP (1 mM), unlike the current recorded for canine colonic myocytes, and also inactivated faster. Inward current from canine colonic ICC was studied with the perforated patch clamp technique. Inward current activated at 10 mV negative to inward current of canine colonic myocytes and peaked 10–30 mV more negative than canine colonic myocytes. The current-voltage relationship showed a distinct hump at negative voltages, and the current was more dependent on holding voltage than its counterpart was in colonic myocytes. A comparison of steady-state inactivation curves between ICC and myocytes showed that at -30 mV only 13% of the peak current (at -80 mV) remained, compared with 55% for colonic myocytes. The hump was sensitive to nickel (40 μM) and insensitive to nifedipine (1 μM). These electrophysiological and pharmacological data suggest the presence of a low voltage-activated T-type-like Ca^{2+} channel current, supporting a role for ICC as pacemaker cells.

In ICC isolated from normal human jejunal circular smooth muscle, a study on inward Ca^{2+} currents also revealed a hump in the current-voltage relationships of the whole-cell current at negative voltages, with a second inward peak around 0 mV, suggesting the presence of both an L-type Ca^{2+} channel current

and a second Ca^{2+} current with a more negative activation possibly T-type-like. However, at very negative holding voltages (−90 mV), a similar but less pronounced hump in the current-voltage relationship was seen in some human jejunal circular smooth muscle cells. Hence, the difference in Ca^{2+} channels in ICC and myocytes in the human jejunum may be quantitative and not qualitative (130).

For ICC to act as pacemakers, they must generate rhythmic changes in membrane conductances. The membrane conductances in newborn BALB/c mice were determined from ICC maintained in culture for at least two days in the presence of the natural ligand for c-Kit, stem cell factor (127). At a holding voltage of −40 mV, voltage clamp experiments showed a rhythmic oscillation in current paralleled by changes in voltage under current clamp. The current appeared to be carried by Cl^- as it was blocked by the Cl^- channel blocker 4-acetamido-4-isothiocyanatostilbene-2,2′-disulfonic acid (SITS, 300 μM) and the reversal potential tracked the Cl^- equilibrium potential. Another component of the rhythmic current with faster kinetics than the Cl^- current was present but was not studied. In contrast, in a study on rhythmic activity in ICC from adult mouse small intestine, rhythmic inward currents were recorded with a frequency of 6/min (128). The current-voltage relationship reversed at +10–20 mV, suggesting a nonselective cation current. Which ionic conductance or conductances underlie the rhythmic changes in whole-cell current is therefore still unclear. Clearly, ionic conductances in ICC, especially in humans, will be a topic of intense investigation in the coming years.

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